Journal of Genetics (2022)101:40
https://doi.org/10.1007/s12041-022-01385-8

RESEARCH NOTE

Spastic paraplegia 51: phenotypic spectrum related to novel homozygous *AP4E1* mutation

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Received 9 March 2022; revised 21 July 2022; accepted 21 July 2022

Abstract. AP-4-associated hereditary spastic paraplegia (HSP), also known as AP-4 deficiency syndrome, is a genetically diverse group of neurologic disorders defined by complex spastic paraplegia. Different forms of AP-4-associated HSP are classified by chromosomal locus or causative gene. Spastic paraplegia 51 (SPG51) is a neurodevelopmental condition that is caused by autosomal recessive mutations in the adaptor protein complex 4 complex subunit 1 (*AP4E1*) gene. Further, previous studies described an autosomal dominant mutation in the *AP4E1* gene has also been linked to persistent stuttering. Here, we describe a patient from a consanguineous marriage who manifested severe intellectual disability (ID), absent speech, microcephaly, seizure, and movement disorders. Exome sequencing identified a novel homozygous frame-shift variant (NM_007347.5:c.3214_3215del, p.Leu1072AlafsTer10) in the *AP4E1* gene, which was confirmed by Sanger sequencing. In this study, we also reviewed the phenotype of the former cases. Our findings added to the knowledge of little-studied homozygous *AP4E1* mutation.

Keywords. *AP4E1* gene; spastic paraplegia 51; whole-exome sequencing.

Introduction

Hereditary spastic paraplegias (HSP) are a genetically and clinically diverse category of more than 80 neurodegenerative disorders that cause progressive neurologic impairment. Most forms of HSP are defined by their genetic loci that are numbered in order of their discovery (Fink 2013). Four types of HSP have recently been found associated with deficiency of different adaptor protein 4 (AP-4) complex subunits (β4, ε, μ4 and σ4). AP-4-associated hereditary spastic paraplegia (AP-4-HSP) is caused by homozygous mutations in genes that encode subunits of the adaptor protein complex 4 (AP-4) (β4, ε, μ4 and σ4): SPG47 (OMIM: 614066, *AP4B1*), SPG50 (OMIM: 612936, *AP4M1*), SPG51 (OMIM: 613744, *AP4E1*), and SPG52 (OMIM: 614067, *AP4S1*). The loss of function of the AP-4 protein complex is the molecular mechanism in all AP-4-HSP disorders (Ebrahimi-Fakhari *et al.* 1993).

AP-4E1-associated spastic paraplegia 51 (SPG51) is an autosomal recessive condition characterized by intellectual disability, speech problems, movement disorders and neurologic problems (Moreno-de-luca *et al.* 2011). *AP4E1* is a protein-coding gene that encodes a member of the adaptor complex's large subunit protein family. AP-4 complex subunit ε − 1 is part of the heterotetrameric adaptor protein complexes, which control the vesicular transport of proteins in various trafficking pathways by being able to recognize a variety of sorting signals. According to the previous research, AP-4E1 facilitates the protein sorting to the basolateral membrane in epithelial cells and is essential in the establishment of efficient somatodendritic protein asymmetric localization in neurons (Hirst *et al.* 1999). The characterization of Ap4e1-knockout mice has revealed a range of neurologic phenotypes that include hind limb clasping and decreased motor coordination (De Pace *et al.* 2018).

Insights into the molecular mechanisms of SPG51 and the pathogenic variants in the *AP4E1* gene could lead to targeted therapeutic options. However, the consequences of a loss of function of *AP4E1* remain to be assessed in patients. In the current paper, we characterize a novel mutation in the *AP4E1* gene, a novel homozygous frame-shift variant that

Published online: 06 September 2022

causes SPG51. Intellectual disability (ID), seizure, speech disorder, and movement disorders have been observed in our case.

Materials and methods

Subject

The proband is a nine-year-old girl who was referred to our centre with movement disorders and developmental delay. She is the first born of consanguineous parents (first cousin). The evaluation in the study is based on a detailed clinical history and past medical records. Written informed consent was obtained from the parents of the patient. Peripheral blood samples were collected from the subject and parents for further investigations.

DNA extraction

Genomic DNA was extracted from the patient's peripheral white blood cells using a QIAamp DNA Blood Mini kit according to the manufacturer's protocol (Qiagen, Hilden, Germany).

Whole exome sequencing (WES) and data analysis

Whole-exome sequencing was performed on the extracted DNA of the white blood cells of the proband using a HiSeq 3000/4000 SBS kit. The raw data were aligned against the human reference genome (hg19) using the Burrows-Wheeler Aligner (Li and Durbin 2010). Single-nucleotide polymorphisms (SNPs) were called by the software program Genome Analysis Toolkit (GATK). Variants were annotated using ANNOVAR (Wang *et al.* 2010). Each variant was classified into one of five categories, namely pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign and benign, based on the American College of Medical Genetics and Genomics (ACMG) standards for the interpretation of sequence variations (Richards *et al.* 2015; Zoghi *et al.* 2021). The phenotypic features associated with the candidate genes were compared with the patient's phenotype (Zoghi *et al.* 2021).

WES identified homozygous variant (NM_007347.5:c.3214_3215del, p.Leu1072AlafsTer10) of *AP4E1* in the proband. Pathogenic variants of *AP4E1* are associated with SPG51, autosomal recessive (SPG51). The core phenotype associated with the identified mutations was obtained from the OMIM database (OMIM: 613744). The frame-shift variant (NM_007347.5:c.3214_3215del, p.Leu1072AlafsTer10) detected by WES in the *AP4E1* is classified as 'pathogenic' based on the PVS1, PM2 and PP3 criteria of the ACMG/AMP guidelines (Richards *et al.* 2015). This variant also was not found in gnomAD genomes and the homozygous allele count was zero. This frame-shift variant in the *AP4E1* gene identified in the present study is not listed in the ClinVar (ncbi.nlm.nih.gov), LOVD (lovd.nl).

In silico predictions

This frame-shift variant (NM_007347.5:c.3214_3215del, p.Leu1072AlafsTer10) is classified as a 'pathogenic' or 'disease-causing' based on some pathogenicity predictions tools such as the combined annotation dependent depletion (CADD), MetaLR, protein variation effect analyser (PROVEAN), LIST-S2 and FATHMM-MKL.

Sanger sequencing

The primers were designed using Oligo Primer Designer. PCR was carried out using specific primers flanking the frame-shift variant (NM_007347.5:c.3214_3215del, p.Leu 1072AlafsTer10) in *APE41* gene. The segregation of the variant was studied in the family via Sanger sequencing (figure 1b). PCR was performed using 25 μL of the Hot start mix Ampliqon, 70 ng of DNA, 1 μL of forward primer (5′-CTTATGGGACCCAGATTTAC), 1 μL of reverse primer (5′-CTGAAAGACAGTCATAATAGG), and 21 μL deionized water. The DNA was amplified using the following Thermocycling steps: 95°C for 15 min; 35 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 15 s; 72°C for 5 min. This variant was confirmed by Sanger sequencing in the proband and her parents. Her healthy parents (father and mother) were heterozygous for this pathogenic frame-shift variant in the 20th exon of *AP4E1* gene. Sanger sequencing with bioinformatics analysis show that this variant is not a polymorphism, but is instead a rare pathogenic variant segregating with autosomal recessive disease in this family.

Case presentation

The proband was a nine-year-old girl who was referred to our centre with movement disorders and developmental delay. She was the only child of consanguineous parents (first cousin) (figure 1a). The girl was born following a successful pregnancy at 38 weeks of gestation and delivered by cesarean section with normal birth parameters. Her weight at birth was 3800 g, her length was 54 cm, and her head circumference was 38 cm. Severe psychomotor retardation and developmental delay were evident in the neonatal period. Examination revealed head lag, poor eye contact and variable tone in the extremities. She was affected with tonic flexion seizures since the second month after birth. Routine growth check-up showed that she had failed to reach developmental milestones. She was able to sit with support at the age of 12 months.

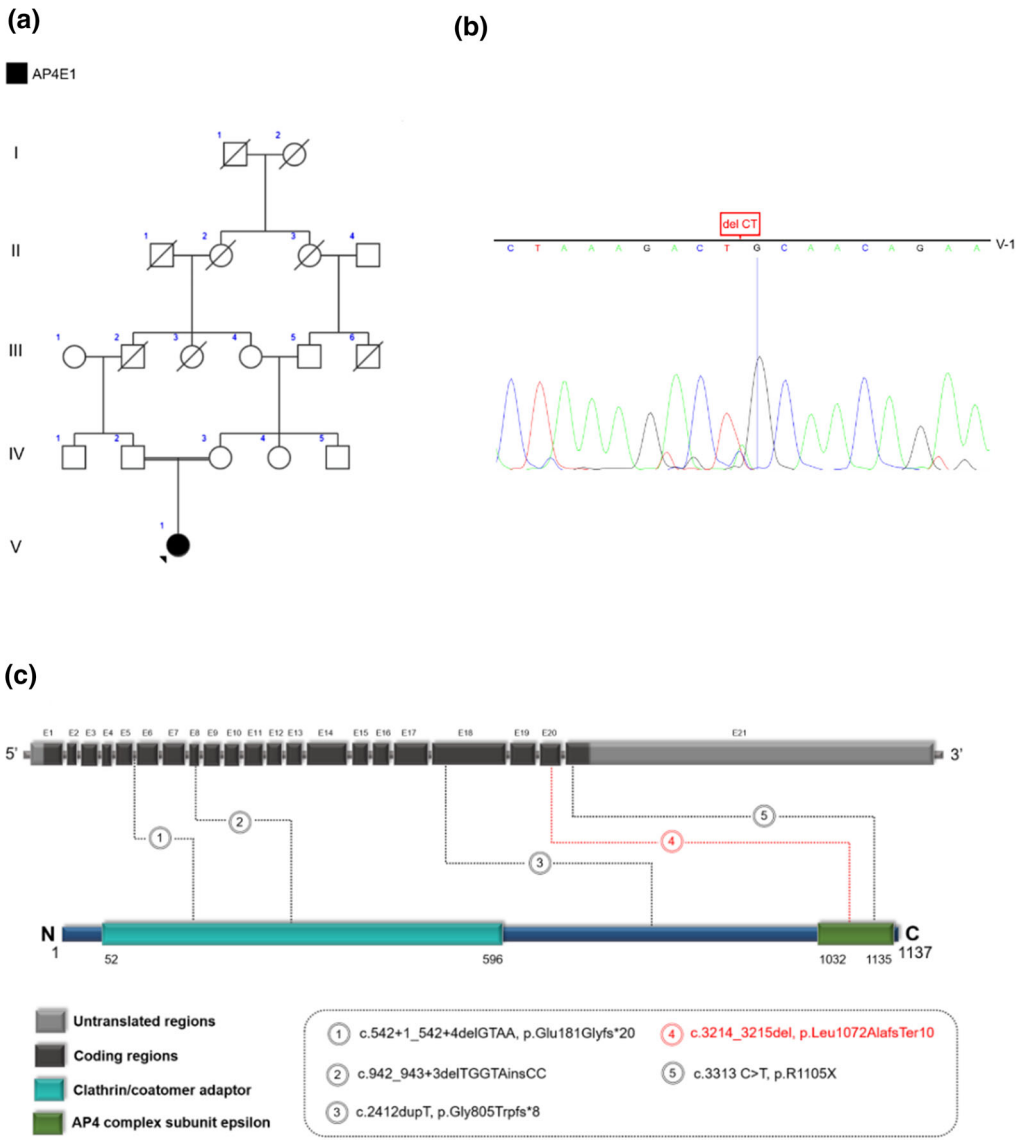

Figure 1. Pedigree, electropherograms of the proband (V-I), and the schematic depiction of AP4E1 protein. (a) Pedigree of the family illustrating the recessive inheritance of the pathogenic variant in this family. (b) Electropherogram of the proband showing pathogenic variant (NM_007347.5:c.3214_3215del, p.Leu1072AlafsTer10) in the *AP4E1* gene. (c) A schematic depiction of the human *AP4E1* locus with 21 exons in the top and AP4E1 protein in the bottom. Dash line indicates pathogenic variants. The red dash line presents the variant in this study.

Following a delay, she started to walk at the age of three-and-a-half years; however, she could not walk independently. She was never able to walk without aid. Spastic diplegia was identified on neurological assessment at 3 years and 7 months of age. The speech delay in this patient was more severe than the previously reported cases of SPG51. She had severe ID, in line with previous reports of SPG51. Ataxia was detectable with a slightly wide-based gait and balance difficulty in the patient. Her arm and leg movements were uncoordinated and restless. Proband had severe postural unsteadiness and tremors. Although treated with phenobarbital and topiramate, she suffered from refractory seizures. Her eyesight is intact and no sign of visual impairment in the field or acuity was recorded.

Written informed consent was obtained from the parents. This study was ethically approved by the Ethics Committee of Shiraz University of Medical Sciences.

Result and discussion

The present paper reported the case of a nine-year-old Iranian girl with significant developmental delay, speech disorder and hypertonia. She was unable to ambulate independently. Her neurological assessment showed severe intellectual disability and hypotonia. She developed her first seizure at the age of two months. Also, similar to the previously reported cases, she manifested microcephaly, short

Table 1. Phenotypic delineation of the affected individuals. A review of the phenotypic features of all previous cases and our patient that reported in this study.

	Family 1		Family 2		Family 3	Family 4		Family 5
	I-1	II-1	I-2	II-2	I-3	I-4	II-4	I-5
Variant	c.542+1_542 +4delGTAA, p.Glu181Glyfs*20	c.542+1_542 +4delGTAA, p.Glu181Glyfs*20	c.3313 C>T, p.R1105X	c.3313 C>T, p.R1105X	c.2412dupT, p.Gly805Trpfs*8	c.942_943 +3delTGGTAinsCC	c.942_943 +3delTGGTAinsCC	c.3214_3215del, p.Leu1072AlafsTer10
Gender	Male	Female	Female	Female	Female	Male	Male	Female
Parents consanguinity	+	+	+	+	+	+	+	+
Age at examination (years)	11	6	Infancy	Infancy	5	12	12	9
Intellectual disability	Severe	Severe	Severe	Severe	Severe	Severe	Severe	Severe
Speech disorders	+	+	+	+	+	+	+	+
Hypotonia	+	+	+	+	+	+	+	+
Spasticity	+	+	+	+	+	+	+	+
Short stature	+	+	+	+	+	+	+	+
Microcephaly	+	+	+	+	+	+	+	+
Inability to walk independently	+	+	+	+	+	+	+	+
Stereotypic laughter	+	+	+	+	+	−	−	−
Foot deformity	+	+	N/A	N/A	N/A	+	+	+
Hypertonia	+	+	−	−	N/A	+	+	+
Drooling	+	+	+	+	+	N/A	N/A	−
Seizures	−	+	−	−	+	−	−	+
Babinski sign	+	+	−	−	N/A	−	−	−
References	Jamra *et al.* (2011)	Jamra *et al.* (2011)	Kong *et al.* (2013)	Kong *et al.* (2013)	Murakami *et al.* (2020)	Winkler *et al.* (2021)	Winkler *et al.* (2021)	Present study

+, Present; −, absent; N/A, not available.

stature, foot deformity, and spasticity. WES was performed, and a disease-causing mutation was found in the *AP4E1* gene, which codes the AP-4 adaptor protein complex subunit ε − 1.

Null variants, in gene *AP4E1* for which loss-of-function (LOF) is a known mechanism of disease, are associated with hereditary SPG51. Several case studies have been published reporting patients with SPG51 due to mutation in *AP4E1* gene. *AP4E1* gene has eight pathogenic LOF variants causing altered gene product which lacks the molecular function.

The phenotypic spectrum arising from disruptions in the *AP4E1* is variable among previously reported cases, but the core clinical phenotype is consistent. The main clinical features observed in the present study are the same as the symptoms observed in the individuals reported in other case reports (Jamra *et al.* 2011; Kong *et al.* 2013; Murakami *et al.* 2020; Winkler *et al.* 2021).

So far, eight patients from five families have been diagnosed with mutations in the *AP4E1* gene (OMIM: 613744). All of the patients were born to consanguineous parents. Five individuals were female and others were male. The features of the previously reported patients with SPG51 and our subject are reviewed in table 1.

Prevalence of each trait among the patients is shown as a ratio of the total cases. In general, the most common presentations among all patients were intellectual disability, speech disorders, microcephaly and hypotonia (8/8). All affected individuals manifested spasticity, short stature and inability to walk independently (8/8). A significant fraction of cases with a pathogenic mutation in the *AP4E1* gene has shown foot deformity, hypertonia, stereotypic laughter, and drooling (5/8). However, stereotypic laughter and drooling were not observed in our subject. On the other hand, although seizures were only observed in a few cases, the patient in this study suffered from seizures since the second month of birth.

The clinical and molecular spectrum of AP4E1 associated SPG51 need further characterization. The main challenge for the future proceeding from the findings of this study is the prenatal detection of the novel identified variant; the next step would involve more researches to define the phenotypic spectrum of SPG51 and detect new variants which could cause symptoms ranging from mild to severe. As a result, early molecular detection may enhance the diagnostic procedure, symptom management, and genetic counselling of affected patients by HSP and their families.

Some features are similar to previously reported cases, while others are not. Reporting these features can refine our understanding of SPG51, an understudied disease that is probably more prevalent than previously assumed. We also provide the first comprehensive review on SPG51, which is quite useful in diagnosing and evaluating new patients with suspected SPG51.

There is currently no treatment for this condition; however, speech therapy and supportive care may help mitigate problems such as speech development and movement abnormalities, among others. Patients should be closely monitored for probable seizures and complications related to seizures. In summary, we believe that symptomatic therapy should be considered as soon as a problem is detected until a more effective treatment is developed.

Iran is part of the consanguinity belt, which stretches through north Africa to south India. This high proportion of tightly consanguineous marriages brings about a group of genetic disorders that are rare individually but collectively abundant (Khamirani *et al.* 2021c, a, b; Faghihi *et al.* 2022; Jafari Khamirani *et al.* 2022a, b). This family with an affected child who inherited homozygous AP4E1 mutation is a good example of this point.

Current evidence is insufficient to resolve the inconsistencies observed in SPG51 patients. We believe that patients with SPG51 should be more rigorously reported. Better characterization of the disorder is likely in the future as more literature is published on SPG51.

Acknowledgements

The authors thank the Comprehensive Medical Genetics Center, Shiraz, Fars Province, Iran, for their invaluable contribution to this study.

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Corresponding editor: Ashwin Dalal